Localization and analysis of engineered nanoparticles in Daphnia Magna

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Engineered nanoparticles are added to a growing amount of consumer products and are considered the fastest growing nanotechnology product. They are being washed out into the waste water systems in potentially increasing amounts as the use and number of products that contain nanoparticles is expanding. There are concerns that they could be harmful to aquatic organisms in nature. The uptake mechanisms in aquatic organisms are therefore important to elucidate the toxic mechanisms.

Previous results have indicated that gold nanoparticles to a limited extent are able to pass the peritrophic membrane and in some cases enter the gut epithelial cells in the freshwater crustacean *Daphnia magna* (ref1). In the absence of food, the amount of gold nanoparticles is elevated compared to when the animal is feeding. It has been speculated that when gold nanoparticles are present in the gut in relatively high amounts they might be able to cross the peritropic or cell membrane during the preparation for electron microscopy, perhaps during the epon infiltration step. In the present study *D. magna* were exposed for 24h to 0.4 mg Au/L citrate coated gold nanoparticles (10 nm) without food and an additional control was added to the experiment; *D. magna* which were exposed to gold nanoparticles during the infiltration process for electron microscopy. The uptake into gut lumen and internalization into epithelial cells of exposed *D. magna* was examined by light microscopy, TEM, FIB-SEM and EDX. The bulk of the gold nanoparticles in the exposed animals was observed as both single nanoparticles and aggregates located in the gut lumen while relatively few gold nanoparticles were observed across the peritrophic membrane associated with the microvilli. This is indicating that the peritrophic membrane of the gut in *D. magna* is able to form a barrier of low permeability to the gold nanoparticles in all cases. Very few gold nanoparticles were found to be internalized by the gut epithelial cells. The results suggest that 10 nm gold nanoparticles are able pass the peritrophic membrane and enter the gut epithelial cells.

Ref1: Lars Michael Skjolding, Sara Nørgaard Sørensen, Amalie Thit, Carsten Købler, Kristian Mølhave, Anders Baun. Uptake of gold nanoparticle in *Daphnia magna* gut in the presence and absence of food using electron microscopy. To be presented at SETAC Europe 24th Annual Meeting, May 2014.

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